A New Approach to Prenatal Diagnosis Using Trophoblast Cells in Maternal Blood*

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Amniocentesis has been extensively used for prenatal diagnosis of certain genetic disorders.[1-4] In spite of its considerable value, this procedure carries definite, though infrequent, fetal and maternal risks. These complications include bleeding, infection, abortion, fetal injuries, fetomaternal sensitization, etc.[5-9] Moreover, therapeutic abortions following amniocentesis are usually induced late in the second trimester and are more risky than those induced earlier in pregnancy.[8]

The aim of the present work is to explore the possibility of using trophoblast cells recovered from the maternal circulation, instead of amniotic cells, for prenatal diagnosis. If this could be done, the complications arising from amniocentesis could be avoided. It would also be possible to induce earlier and less risky abortions, since human trophoblast cells start to deport into the maternal circulation early in the first trimester of pregnancy.[10-12]

The following is a short report on the studies performed to evaluate this approach in experimental models. The details of this work will be published separately.[13-15]

Experiment I

This experiment was designed to test the efficiency of our method for detection and recovery of trophoblast cells suspended in the maternal blood.

Healthy human placental tissue was obtained from early abortions and cut into tiny pieces in petri dishes containing a few milliliters of TC 199 medium. The trophoblast-199 suspension was then mixed with a 20 ml sample of heparinized peripheral blood taken from the same patient. The resulting trophoblast-blood suspension, containing known amounts of trophoblast cells (Fig. 1A), was then divided into 2 tubes which were allowed to stand in the refrigerator for 4 hours and 24 hours, respectively. Smears were then made from the supernates, buffy coats, and red cell sediments of both tubes (Figs. 1B and C). The remaining buffy coat was kept for the tissue culture studies (**Experiment II**). The smears were stained with Giemsa stain and examined for the number and condition of the trophoblast cells and leukocytes, and for the amount of RBCs in the background.

Sixty-one percent of the suspended trophoblast cells were recovered from the buffy coat after 4-hour sedimentation and 82% after 24 hours. The recovered trophoblasts were seen as well-preserved single cells (Fig. 2A) and as small groups of cells (Fig. 2B), which were easily identified by their large size and tendency towards multinucleation. In addition, syncytial

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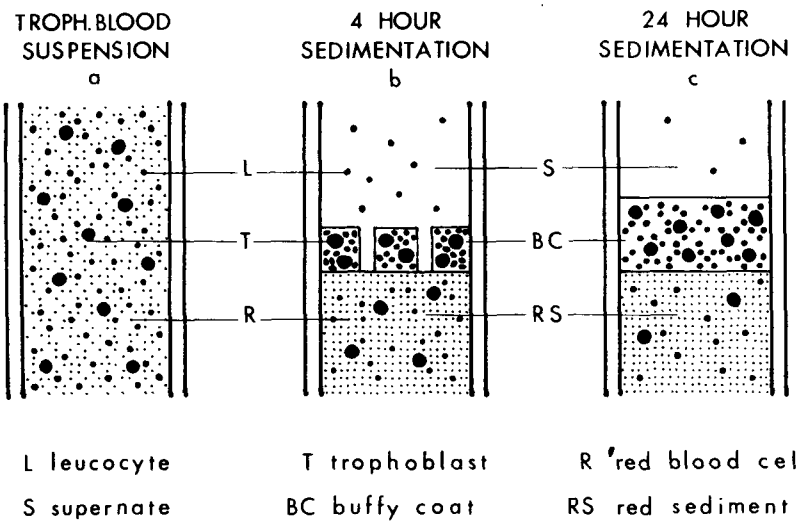

Fig. 1. Diagram showing the methods used for recovery of the trophoblast cells from trophoblast-blood suspension. A) Trophoblast-blood suspension before sedimentation. B) After 4-hour sedimentation, notice the patchy buffy coat. C) After 24-hour sedimentation, notice the complete buffy coat.

masses (Fig. 2C) and even big portions of chorionic villi (Fig. 2D) were also seen. The 24-hour sedimentation method was preferred to the 4-hour method since it yielded more trophoblast cells and leukocytes, and showed fewer RBCs in the background.

Experiment II

This experiment was designed to compare the growth potentials in vitro of the trophoblast cells recovered from the maternal blood suspension (**Experiment I**), with those directly explanted from the placenta of the same patient.

Trophoblast cells, obtained from both sources, were cultured in T30 plastic flasks. Half the flasks were lined with thin layers of reconstituted rat-tail collagen, which was prepared following a modification of the technique of Ehrmann and Gey.[16] Three culture media were used: TC 199 with 33% fetal bovine serum (FBS), Eagle's MEM with 15% FBS, and Gibco diploid growth medium with 10% FBS. The cultures were incubated at 37°C in a gas phase of 5% CO_2 in air. They were periodically examined for cell morphology, growth rate and the sequence of events in culture.

In all the systems used, trophoblast cells grew actively while leukocytes did not grow and gradually degenerated. Apart from a slight delay in the initial migration, the blood-recovered trophoblast cells behaved in a similar manner to those directly explanted from the placenta. The cells grew better on the collagen substrate than on the plastic surface. The 3 media supported the growth of trophoblast cells equally well. The third medium, however, was preferred because of its lower serum content.

About 80% of the explants produced pure epithelial monolayers of trophoblast cells which grew actively for about 4 weeks (Fig. 3A). During this phase of active growth, many mitotic figures were seen (Fig. 3B). Numerous microvilli were also noticed projecting from the free borders of the cells (Fig. 3C). After 4-6 weeks small gaps started to appear in the monolayers (Fig. 3D) and gradually expanded. This was accompanied by progressive degeneration of the trophoblast cells.

About 20% of the explants produced a mixed population of trophoblast and fibrospindle cells. Sometimes a few histiocytes were also seen. These mixed cell cultures were kept growing for about 20 weeks before they started to degenerate. They were transferred several times with gradual disappearance of the trophoblast cells and overgrowth of the fibroblasts.

Collagen lysis was frequently observed, particularly in the mixed cell cultures. Cytogenetic studies revealed a high incidence of polyploidy (20%) mainly in the 4n range. Satisfactory karyotypes were made from both types of cultures (Fig. 4).

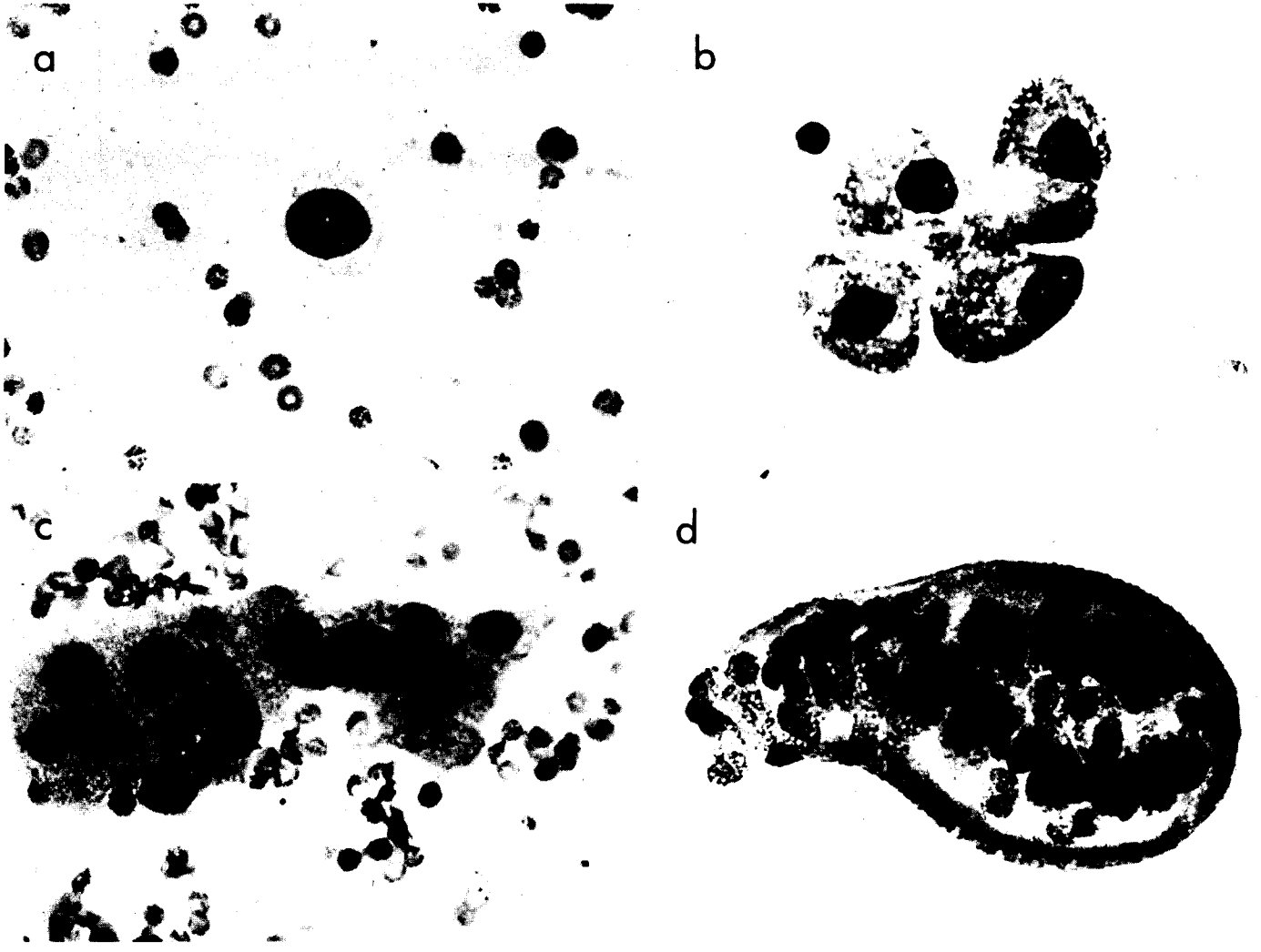

Fig. 2. Morphologic patterns of the recovered trophoblast. Smear stained with Giemsa. Notice the well-preserved condition of the trophoblast cells and their large size as compared to the leukocytes and RBCs in the background. A) A single cytotrophoblast cell (×320). B) A group of cytotrophoblast cells (×320). C) A mass of syncytotrophoblast (×320). D) A big portion of a primary chorionic villus (×160).

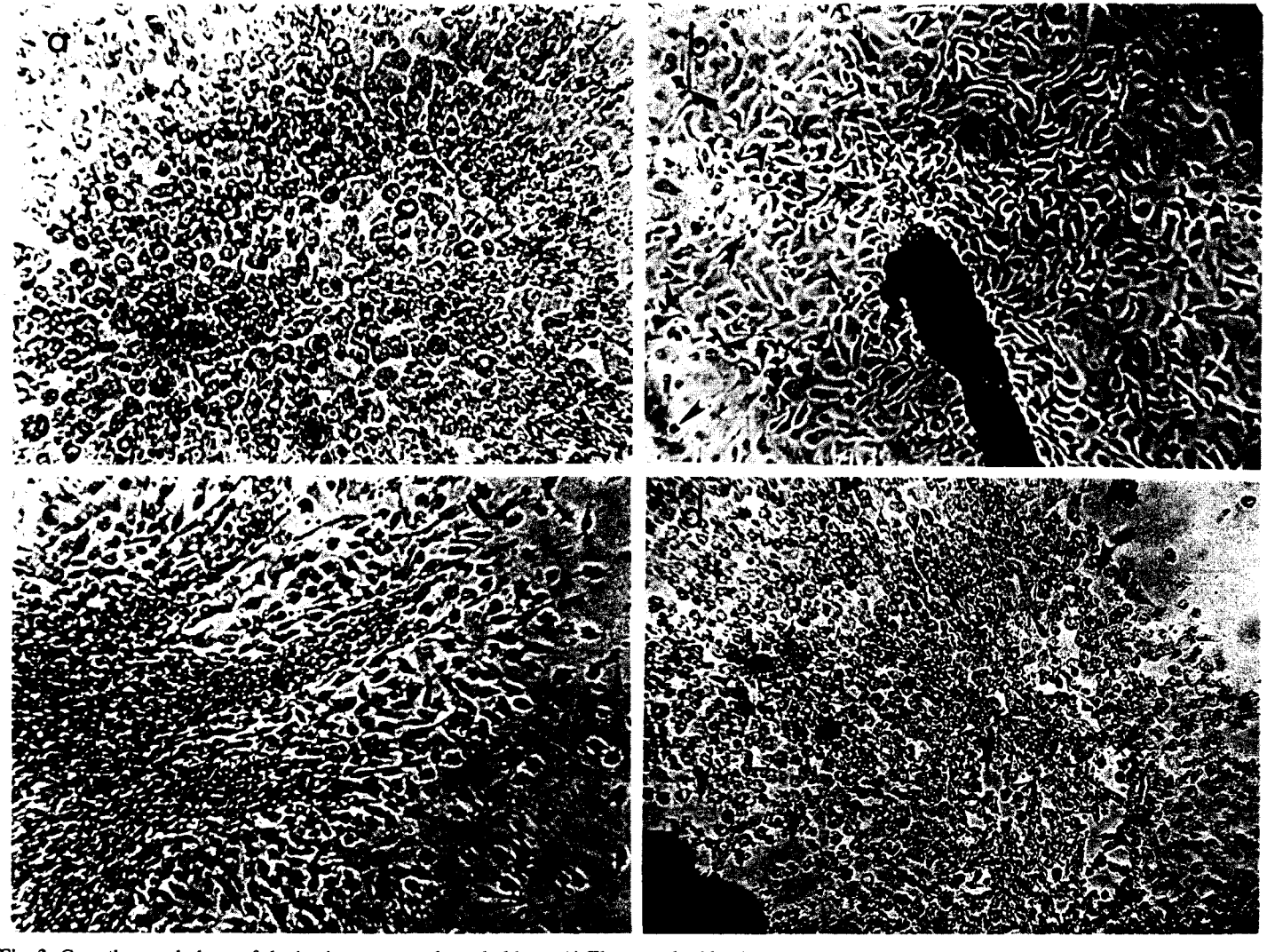

Fig. 3. Growth morphology of the in vitro recovered trophoblasts. A) Three-week-old culture showing an extensive monolayer of trophoblast cells (×90). B) Two-week-old culture showing a large number of dividing cells (×60). C) Three-week-old culture showing microvilli projecting from the free borders of trophoblast cells (×60). D) Five-week-old culture, showing small gaps in the trophoblast monolayer (×60).

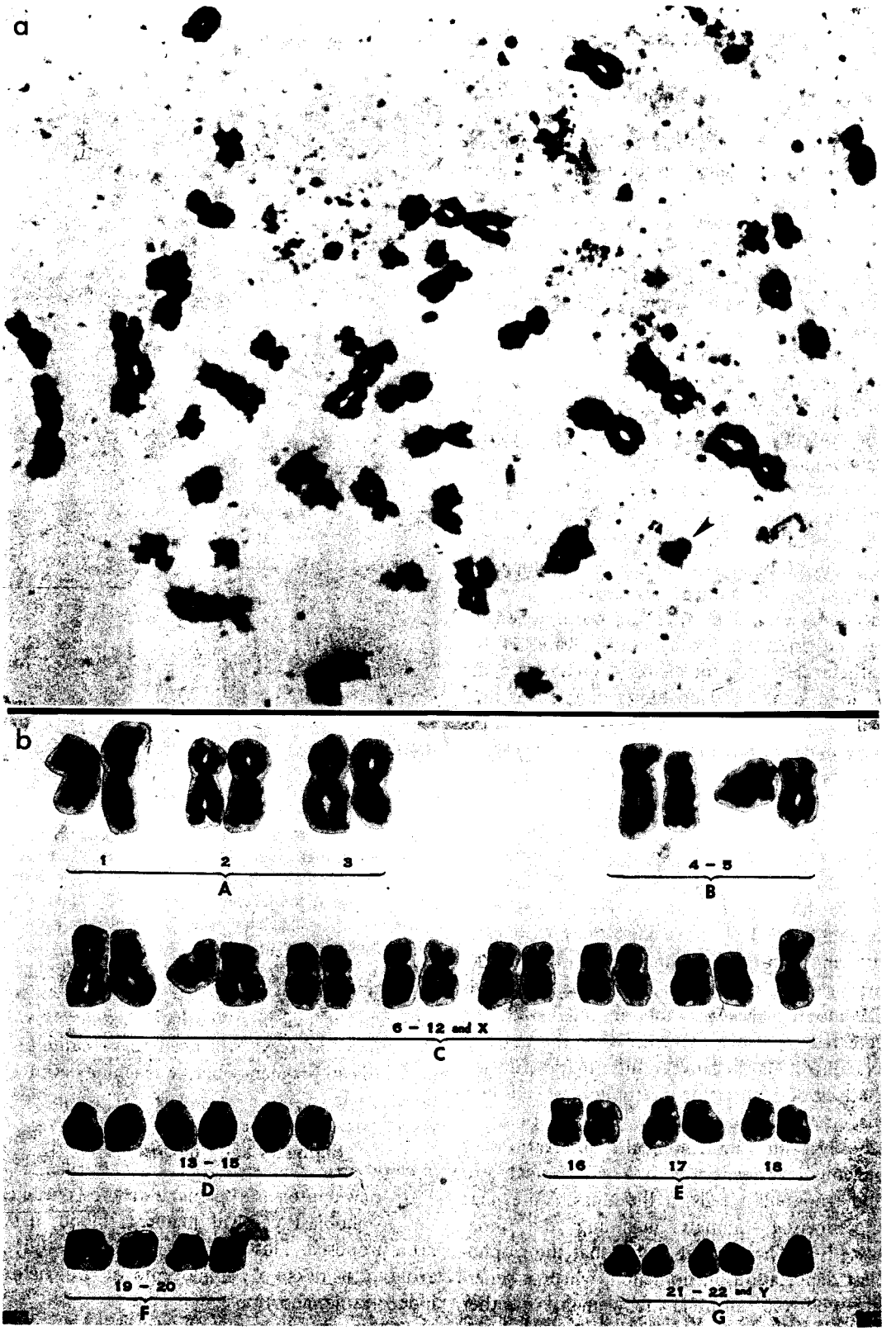

Fig. 4. Chromosomal preparation from a 2-week-old trophoblast culture. Giemsa stain. A) Metaphase spread showing 46,XY. The arrow points to the Y chromosome. B) Karyotype of the same spread.

Experiment III

This experiment was designed to recover the trophoblast cells from the blood circulation of pregnant rhesus monkeys (*Macaca mulatta*). Due to the large size of these cells, they are filtered in the pulmonary capillaries and do not reach the peripheral circulation.[11,17,18] Therefore, for successful recovery, blood samples should be obtained along the venous drainage of the placenta, somewhere between the uterus and the lungs. The higher we go into the inferior vena cava, the more complete will be the sampling, since the drainage of the ovarian veins will be included.[17-19]

The monkeys were given a mild sedative 15 minutes before catheterization. Then by simple venipuncture, a thin radiopaque catheter was introduced into the femoral vein, along the inferior vena cava until its tip reached above the left renal vein (Fig. 5). There was no need for general anesthesia, x-ray confirmation of the catheter level, or for an open incision on the femoral vein. A 10 ml blood sample was then drawn using a heparinized syringe. Attempts were made to recover and grow the trophoblast cells which might be present in the sample, following the methods mentioned in **Experiments I** and **II**. Control samples were taken from the peripheral blood of the pregnant monkey and the male partner.

So far 10 pregnant monkeys have been catheterized: 4 in the second month of gestation and 6 in the third and fourth months. The total gestation period in the rhesus monkey is 5½ months. No trophoblast cells could be detected in either the smears or the cultures examined. The negative results may be due to the lack of deportation of trophoblast cells into the maternal circulation at the time of sampling, thus confirming the observations of previous investigators who were also unable to find trophoblast cells in the lungs of pregnant experimental animals including the monkey.[20,21] Therefore it seems that the trophoblast deportation phenomenon, which is generally observed in pregnant women, is rather specific to the human and does not occur in the monkey. This is possibly related to the structural differences, which are known to exist between the placentas of the 2 taxa.[23] However, more monkeys will need to be catheterized early in pregnancy before reaching definite conclusions.

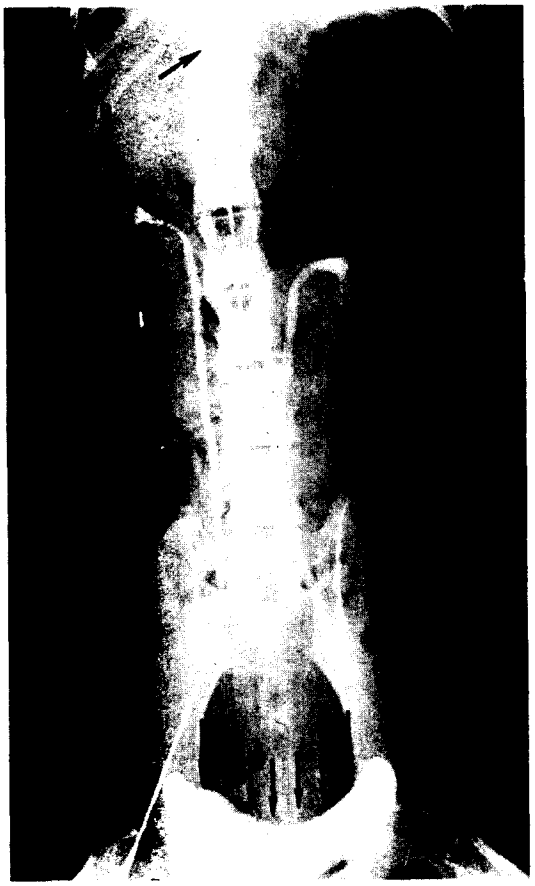

Fig. 5. An IVP of a 46-day pregnant rhesus monkey with the catheter in the inferior vena cava. The upper arrow points to the tip of the catheter. The middle arrow points to the level of left renal vein. The lower arrows point to the indentation in the bladder caused by the pregnant uterus.

Summary and Conclusions

A new approach to prenatal diagnosis, using the trophoblast cells in maternal blood, has been proposed. This approach would hopefully avoid the risks of amniocentesis and help induce earlier abortions.

Three experiments were performed for the evaluation of this approach. In the first experi-

ment human trophoblast cells were successfully recovered from a trophoblast-blood suspension by slow cold sedimentation. In the second experiment, the recovered human trophoblast cells were successfully grown in culture for several weeks and satisfactory karyotypes were prepared from them. In the third experiment attempts were made to recover the trophoblast cells from the inferior vena caval blood of pregnant monkeys, but were not successful. The absence of trophoblast cells in catheter specimens is probably due to the lack of their shedding into the maternal circulation of the monkey.

We recommend trying this new approach in pregnant women since it proved to be simple, safe and efficient in experimental models. Moreover, the trophoblast deportation phenomenon seems to be specific to the human placenta.

* * *

This chapter is dedicated to the memory of Dr. Virginia Apgar, who died during the preparation of the manuscript. Without her inspiration and continuous support, this work would not have been possible.

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